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(54) Title: METHOD OF DETECTING TISSUE-SPECIFIC FK506 BINDING PROTEIN MESSENGER RNAs AND USES THEREOF (57) Abstract <p>A novel mRNA encoding FK506 binding protein (FKBP12) has been identified in human T lymphocytes. The cDNA corresponding to this mRNA has been sequenced and characterized. The mRNA has been shown to be identical in sequence in two regions to two previously known FKBP-encoding sequences and clearly distinct from the two known sequences in the 3' untranslated region (3'UTR). The tissue-distribution of this mRNA also differs from the tissue distribution of the two known mRNAs. DNA probes derived from this transcript encoding a distinct 3'UTR provide a means to detect FKBP12 mRNA in a tissue-specific manner and to use tissue-specific FKBP12 mRNA to monitor rejection of transplanted tissue, and the effects of FK506 immunosuppressant therapy. Nucleic acid sequences which hybridize to these 3'UTRs may also provide a means to modify <i>in vivo</i> production of tissue-specific FKBP mRNA.</p>		

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METHOD OF DETECTING TISSUE-SPECIFIC FK506 BINDING
PROTEIN MESSENGER RNAs AND USES THEREOF

Background of the Invention

5 FK506 and cyclosporin A (CsA) are potent
immunosuppressants that block the early steps of T
lymphocyte activation. These immunosuppressants
interfere with the Ca^{2+} -dependent signaling pathways
mediated by the T cell receptor. (Tocci, M.J. et al.,
10 J. Immunol. 143:718-726 (1989); Mattila, P.S. et al.,
EMBO J. 9:4425-4433 (1990); Bierer, B.E. et al., Proc.
Natl. Acad. Sci. USA 87:9231-9235 (1990). This common
biological property is strikingly unpredictable from
the distinct chemical structures of the two drugs, but
15 can be rationalized by the recent finding that
calcineurin, a Ca^{2+} - and calmodulin-dependent
serine/threonine phosphatase (Klee, C.B. and Krinks,
M.H. Biochemistry 17:120-126 (1978)), is a common
target of these immunosuppressants when they are bound
20 to specific intracellular receptors in vitro and in T
lymphocytes. (Liu, J. et al., Cell 66:807-815 (1991);
Friedman and Weissman, 1991). These receptors, termed
immunophilins, apparently play key roles in
immunosuppressive mechanisms and potentially offer
25 insight into general cytoplasmic signaling and protein
transport mechanisms within cells. (McKeon, F. Cell
66:823-826 (1991).

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FKBPs (FK506-binding proteins) are members of a class of immunophilins that form complexes with FK506, as well as with the structurally related immunosuppressive macrolide rapamycin. (Schreiber, S.L. Science 251:283-287 1991). Both FK506 (Harding, M.W. et al., Nature 341:758-760 (1989; Siekierka, J.J. et al., Nature 341:755-757 (1989; Harrison, R.K. and Stein, R.L. Biochemistry 29:3813-3816 (1990) and rapamycin (Bierer B.E. et al., Proc. Natl. Acad. Sci. USA 87:9231-9235 (1990) inhibit the peptidyl-prolyl isomerase activity of FKBPs. The first FKBP to be described at the protein (Harding, M.W. et al., Nature 341:758-760 (1989; Siekerka, J.J. et al., Nature 341:755-757 (1989) and cDNA (Maki, N. et al., Proc. Natl. Acad. Sci. USA 87:5440-5443 (1990; Standaert, R. F. et al., Nature 346:671-674 (1990) levels was FKBP12, an abundant cytosolic immunophilin of M_r 11,800. More recently, FKBP13 (Jin, Y.J. et al., Proc. Natl. Acad. Sci. USA 88:6677-6681 (1991)) and FKBP25 (Galat, A., et al., Biochemistry 31:2427-2434 (1991) have been characterized as immunophilins that bind both FK506 and rapamycin. All of these FKBPs share a core consensus sequence with FKBP12 but only the FKBP12-FK506 complex inhibits calcineurin. This finding implies that the different FKBPs are likely to have diverse effects on signal transduction pathways even though their immunosuppressant binding properties reflect aspects of structural similarity.

The significant immunosuppressive activity of the FKBP12-FK506 complex makes it one of several drugs of choice for prophylactic therapy in transplant patients to prevent tissue rejection (Todo, S., et al., Ann Surg. 212:295-307 (1990); Fung, J. J. et al., Transplant. Proc. 23:2977-2983 (1991)).

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Summary of the Invention

The present invention relates to the identification and characterization of a novel messenger RNA (mRNA) encoding an FK506 binding protein in human T lymphocytes and to the sequencing of its corresponding cDNA (SEQ ID NO:1). Sequencing of the cDNA reveals that the mRNA, also referred to as the RNA transcript, is identical in sequence in two regions (i.e., the 5' untranslated region (5'UTR) and the open reading frame region (ORF)) to two known FKBP12-encoding RNA sequences and clearly distinct from the two known sequences in the 3' untranslated region (3'UTR). Furthermore, the tissue distribution of the mRNA of the present invention, which is expressed predominantly in heart, placental and pancreatic tissue, differs from the tissue distribution of the other two mRNAs.

The present invention also relates to isolated DNA which is indicative of tissue-specificity FK506 binding protein mRNAs, and to DNA or RNA probes for use in the detection of nucleic acid sequences (mRNA and DNA) encoding FK506 binding protein mRNA present in specific tissue.

This invention further relates to a method of detecting the expression of tissue-specific FKBP12 mRNA as a means of monitoring tissue rejection in transplant patients. Thus far, indication of tissue rejection has been diagnosed by a combination of clinical, biochemical and pathological criteria. These present methods are complicated, time consuming, and still do not allow early detection. (Todo, S. et al. Ann. Surg. 212:295-307 (1990), Fung, J.J., et al. Transplant. Proc. 23:2977-2983 (1991)). For example, the diagnosis of liver allograft rejection is based on clinical manifestation of fever without a source of infection,

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bil output or increased ascites; the biochemical indices of total bilirubin, SGOT, SGPT, alkaline phosphatase and other enzyme levels; and the pathological and histological evidence of tissue inflammation unique to tissue rejection situations.

In a preferred embodiment, RNA from tissue of a human is combined with a DNA or RNA probe which is complementary to all or a portion of the RNA sequence of the 3'UTR of a mRNA encoding FKBP12, the cDNA is shown in SEQ ID NO:2. Detection of hybridization is an indication of the presence of a mRNA encoding FKBP12 in specific tissue. The extent to which FKBP12 is produced can also be determined by western blot analysis of proteins purified from individual tissues.

The present method is particularly advantageous because it makes it possible to detect tissue rejection due to an ineffective dosage of FK506 at an earlier stage than is possible with other available methods and, thus, to modify the drug dosage to increase the chances of tissue survival.

The present invention also relates to a method of determining the effects of FK506 therapy on an individual. Clinical management of immunosuppressive therapy treads a fine line between maintaining the optimal drug level which avoids rejection of the transplanted tissue, while minimizing the adverse effects associated with immunosuppressant drugs. Some of these adverse side effects include nephrotoxicity, malignant complications (e.g., posttransplant lymphoproliferative disease), alterations in glucose metabolism and neurotoxicity.

Therefore, it is critical to maintain stringent regulation of FK506 therapy, both in terms of over-all dosage and bioavailability to the specific tissues involved. RNA which is present in specific

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tissue of a human can be hybridized to a DNA or RNA probe which is complementary to all or a portion of the RNA sequence of the 3'UTR of an FKBP12 mRNA, the cDNA is shown in SEQ ID NO:2. Detection of hybridization is indicative of mRNA present which encodes FKBP12, and in what amount. The amount of FKBP12 mRNA present is a reasonable indication of the amount of FKBP12 being produced in the target tissue. If insufficient FKBP12 is produced to be effective in immunosuppressive therapy, another line of therapy may be initiated before rejection or toxicity occurs.

The present invention also relates to a method of modifying production of FKBP12 in vivo. In the method, a synthetic oligonucleotide which hybridizes to all or a portion of the 3'UTR of an FKBP12 mRNA can be administered to an individual. The hybridization of the nucleotide sequence could result in modification of the production of FKBP12. In a preferred embodiment, the nucleotide sequence administered will be a DNA sequence, such that when it hybridizes to the 3'UTR of an FKBP12 mRNA translation will be modified.

Brief Description of the Drawings

Figure 1 shows the FKBP12A nucleic acid sequence. (SEQ ID NO: 1).

Figure 2 shows the FKBP12A 3' untranslated region (3'UTR) sequence. (SEQ ID NO: 2).

Figure 3 shows the comparison of the FKBP12A, FKBP12B and FKBP12C, including their respective 3'UTRs. (SEQ ID NOS: 1, 3 and 4 respectively).

Figure 4 shows the electrophoretic gel pattern of human genomic DNA digested with one of five

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different restriction endonucleases and then used for Southern analysis.

Figure 5 is a schematic representation of the human FKBP12 gene which spans approximately 17-30 kb and contains five exons that encode three different FKBP12 mRNAs.

Figure 6 is a schematic representation of three FKBP12 mRNAs (12A, 12B, 12C) encoded by the FKBP12 gene (SEQ ID NOS:14-25).

Figure 7A shows results of Northern analysis which indicates that poly(A)+RNA from E⁺T cells enriched from human PBLs contains FKBP12A (0.9 kb), FKBP12B (1.7 kb), and FKBP12C (0.7kb) mRNA transcripts.

Figure 7B shows PCR analysis results of first-strand cDNA transcribed from cytoplasmic RNA and demonstrates that the FKBP12A and FKBP12B mRNAs are produced in an enriched, PHA-stimulated population of T lymphocytes.

Figure 8A shows poly(A)+RNA isolated from enriched populations of human E⁺T cells that were stimulated for 0, 4, 16, or 48 hr with 10 $\mu\text{g ml}^{-1}$ PHA.

Figure 8B shows that a 72 hour exposure of the membrane used in 8A reveals that PHA stimulation also increases the abundance of the 0.9 kb FKBP12A mRNA transcripts.

Figure 9 depicts the electrophoretic gel pattern demonstrating that the 1.7 kb FKBP12 mRNA transcript is present in a variety of human tissues.

Figure 10 is a schematic representation showing the synthetic oligonucleotides and restriction enzyme cleavage sites used to generate probes specific to the different FKBP12 cDNAs and mRNA and the FKBP12 gene (SEQ ID NOS:8-11 and 26-33).

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Detailed Description of the Invention

The present invention relates to the identification and characterization of a novel messenger RNA (mRNA) encoding an FK506 binding protein in human T lymphocytes and the sequencing of its corresponding cDNA. Sequencing of the corresponding cDNA revealed that the mRNA, or RNA transcript, is identical in sequence in two regions (i.e., the 5' untranslated region (5'UTR) and the open reading frame region (ORF)) to two known FKBP12-encoding RNA sequences and clearly distinct from the two known sequences in the 3' untranslated region (3'UTR). Furthermore, the tissue distribution of the mRNA of the present invention, which is expressed predominantly in heart, placental and pancreatic tissue, differs from the tissue distribution of the other two mRNAs.

The present invention more particularly relates to the cDNA clone containing an open reading frame that encodes a specific FK506 binding protein hereinafter referred to as FKBP12A (SEQ ID NO: 1), as shown in Figure 1.

As described in detail in Example 1, oligonucleotide probes specific to the FKBP12 open reading frame (ORF) sequence (Maki, N. et al., Proc. Natl. Acad. Sci. USA 87:5440-5443 (1990); Standaert, R.F. et al., Nature 346:671-674 (1990) were used to screen T cell cDNA libraries for other full-length FKBP12 cDNAs. Two different human T lymphocyte cDNA libraries yielded several candidate cDNA clones, and DNA sequence analysis indicated that one of the clones contained the complete FKBP12 ORF. Further analysis showed that the polyadenylated cDNA has a 5'UTR and ORF sequence identical to the cDNAs reported by Maki, N. et al., Proc. Natl. Acad. Sci. USA 87:5440-5443 (1990) and Standaert, R.F. et al., Nature 346:671-674 (1990),

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and a 3'UTR distinct from those of the previously known FKBP12 cDNA clones (Figures 2 SEQ ID NO: 12, and 3 SEQ ID NOS: 1, 3 and 4). The newly cloned cDNA was designated FKBP12A (SEQ. ID NO: 1) and the others
5 designated as FKBP12B (SEQ ID NO: 3) (Maki, N. et al., Proc. Natl. Acad. Sci. USA 87:5440-5443 (1990) and FKBP12C (SEQ ID NO: 4) (Standaert, R.F. et al., Nature 346:671-674 (1990)). FKBP12A is 738 base pairs in length and is predicted to encode a protein of 108
10 amino acid residues (SEQ ID NO: 5). This protein sequence is identical to the deduced amino acid sequence of FKBP12 based on two published FKBP12 cDNA sequences. (Standaert, R.F., et al. Nature 346:671-674 (1990); Maki, N. et al., Proc. Natl. Acad. Sci. USA
15 87:5440-5443 (1990).

As described in detail in Example 2, Southern blot analysis of human genomic DNA was used to determine whether a single FKBP12 gene could encode the FKBP12 mRNAs predicted by the different cDNAs.
20 Radiolabeled DNA fragments specific to the three FKBP12 cDNAs were used as hybridization probes against human genomic DNA cut to completion with one of five restriction endonucleases. Autoradiography revealed a single band in each fractionated digestion when a
25 fragment specific to FKBP12A was used as the probe. (Figure 4, Lane 1 of each digest). Conversely, multiple bands were present when a sequence specific to FKBP12B and FKBP12C was used as the probe (Figure 4, Lane 2). A fragment containing the entire ORF of the
30 cDNAs also produced these multiple bands (Figure 4, Lane 3), with variations only in the Hind III and Pst I digestions. Because the single bands visualized by the FKBP12A-specific probe were contained within each pattern generated by the other probes, it is reasonable
35 to believe that the genomic sequences encoding the

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different transcripts are linked. The simplest interpretation of these data suggests that a single FKBP12 gene encodes the different FKBP12 mRNAs and that two or more FKBP12 pseudogenes are specific to the ORF, FKBP12B, and FKBP12C sequences. This interpretation agrees with the report by DiLella, A.G. and Craig, R.J. Biochemistry 30:8512-8517 (1991) that the human FKBP12 gene has four related pseudogenes. As described in Example 3, the FKBP12 gene was then isolated and characterized. Its location was mapped to chromosome 20.

As described in Example 4, Northern hybridization and PCR analysis were used to identify the FKBP12 mRNAs transcribed from the FKBP12 gene. Hybridization analysis revealed that all three FKBP12 mRNAs are transcribed in erythrocyte receptor positive peripheral blood lymphocytes (E⁺PBL's) (Figure 7A). The FKBP12A transcripts were best detected with a 3'UTR probe, presumably because they are less abundant than the FKBP12B and FKBP12C transcripts which compete for an ORF probe. Additionally, FKBP12B transcripts were made visible by autoradiography in half the time required to visualize the FKBP12C transcripts, presumably due to their relative abundance within the cells. These results suggest that the transcript abundance is FKBP12B>FKBP12C>FKBP12A. This interpretation is consistent with the finding that antisense RNA probes were required for efficient detection of the FKBP12A and FKBP12C mRNAs. The FKBP12A 3'UTR probe also hybridized to a 4.7 kb transcript population (Figure 7A, Lane 1) and, to a lesser degree, to two larger transcript populations (approximately 5.7 kb and 7.8 kb, visible in Figure 7A, Lane 2). These could represent cross-hybridizing mRNAs

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unrelated to FKBP12 production or precursor populations of mature FKBP12 transcripts.

PCR analysis of first-strand cDNA demonstrated that cytoplasmic RNA of T lymphocytes stimulated with PHA (Figure 7B) contains FKBP12A and FKBP12B transcripts. The amplified FKBP12A sequences were consistently less abundant than the amplified FKBP12B sequences (Lane 2 and 3 in Figure 7B), corroborating the results of Northern analysis. Because FKBP12C is a subset of the FKBP12B sequence, internal primers could not distinguish the two transcript populations in PCR analysis.

Northern analysis also indicated that PHA stimulation of E⁺T lymphocytes increased the cellular abundance of the FKBP12A and FKBP12B transcripts (Figure 8). The RNA isolated from enriched populations of human E⁺T cells (5 µg per lane) was fractionated and probed with a synthetic DNA oligomer specific to 63 nucleotides of the FKBP12 ORF. A 16 hour exposure of the probed membrane reveals that PHA stimulation increases the abundance of the 1.7 kb FKBP12B transcripts. The first two time points examined after stimulation with 10 µg ml⁻¹ PHA, 4 hour and 16 hour, showed a progressive increase in these mRNA levels. The third time point examined, 48 hour, revealed an effect similar to that seen at 16 hour. It is reasonable to use standard laboratory techniques as described above, to determine whether the stimulatory effect could be detected prior to 4 hours and whether the effect was linearly or cyclically progressive. Additionally, hybridization experiments using antisense RNA probes rather than DNA probes would reasonably demonstrate whether FKBP12C transcripts also increase in abundance upon mitogenic stimulation.

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Northern analysis demonstrated that the FKBP12 transcripts predominate in poly(A)+RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. As shown in Figure 9 each lane contained 2 μ g of poly(A)+RNA from human tissue: H = heart, B = brain, Pl = placenta, Lu = lung, Li = liver, SM = skeletal muscle, K = kidney, Pa = pancreas. The radiolabeled probe was the DNA oligomer described in Figure 8. Positions of RNA molecular weight markers are shown in kb.

Two different probes both indicate the following approximate relative abundance of FKBP12B transcripts: heart=brain=placenta=lung>skeletal muscle=kidney>liver=pancreas. Heart, placental, and pancreatic tissue appear to contain the FKBP12A transcript (faint bands at 0.9 kb in Lanes H, Pl, and P of Figure 9). Heart and placental tissue also contained uncharacterized, faintly hybridizing RNAs around 4.2 kb.

The three FKBP12 transcripts are produced from a single gene that contains five exons (Figures 5 and 6 (SEQ ID NOS:14-25)) and is located on chromosome 20 (DiLella, A.G. and Craig R.J., Biochemistry 30:8512-8517 (1991)). The four intron-exon splice junctions contain the conserved elements characteristic of eukaryotic genes (Figure 5) and FKBP12 is encoded by the first four exons. The fifth exon provides a 3'UTR sequence exclusive to the FKBP12B and 12C transcripts. The four protein-coding exons correspond to the FKBP12 sequence as follows: Exon 1 encodes amino acids 1-11, Exon 2 encodes amino acids 13-27, Exon 3 encodes amino acids 29-65, and Exon 4 encodes amino acids 66-107 (arrows in Figure 3 designate exon junctions). Amino acids 12 and 28 are encoded by codons that are split by exon boundaries. There is a correlation between the

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exon-encoded domains of FKBP12 and the structural motifs of the protein (Moore, J.M. et al., Nature 351:248-250 (1991) and DiLella, A.G. and Craig, R.J. Biochemistry 30:8512-8517 (1991)). Structural studies
5 of uncomplexed FKBP12 (Michnick, S.W. et al., Science 252:836-839 (1991); Moore, J.M. et al., Nature 351:248-250 (1991) and the FKBP12/FK506 complex (Van Duyne, G.D. et al. Science 252:839-842 (1991) reveal a common hydrophobic pocket that is defined by residues of the
10 five-stranded antiparallel β -sheet on one face and by residues from a loop and single short helix on the opposite face. The residues that comprise the two central strands of the β -sheet are encoded by Exon 4, and the amino acids that constitute the loop and
15 helical region are encoded by Exon 3. X-ray crystallographic studies of the FKBP-FK506 complex reveal the hydrophobic pocket as the protein site that binds the immunosuppressant (Van Duyne, G.D. et al., Science 252:839-842 (1991), thus demonstrating that the
20 residues encoded by Exons 3 and 4 also correspond to domains of function within FKBP12.

The unique 3'UTRs also suggest that stability, translatability, cellular location, and tissue specificity may affect FKBP12 production.
25 (Jackson, R.J. and Standaert, N., Cell 62: 15-24 (1990); Lipshitz, H.D., Current Opinion in Cell Biology 3:966-975 (1991); Leff, S.E. and Rosenfeld, M.G., Ann. Rev. Biochem. 55:1091-1117 (1986)).

The general phenomenon of pseudogenes is well
30 documented for a variety of eukaryotic genes. (Leff, S.E. et al., Ann. Rev. Biochem. 55:1091-1117 (1986). Poly(A) tails and 3'UTR sequences modulate mRNA half-life and translation efficiency in vivo (Jackson, R.J. and Standart, N. Cell 62:15-24 (1990)).
35 Therefore, transcript diversity generated by these

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elements is reasonably viewed as a means to control the final production level of FKBP12 in cells.

Additionally, 3'UTRs are known to regulate spatial expression of proteins by controlling proper transcript

5 localization within cells (Lipshitz, H.D. Current Opinion in Cell Biology 3:966-975 (1991)). It is reasonable to predict that regulated sequestration and translation of FKBP12 transcripts function to position FKBP12 near specific, localized cellular receptors to
10 effect determined responses.

Finally, alternative 3'UTRs of otherwise identical messages also provide a means to control developmental- or tissue-specific expression of proteins (Leff, S.E. et al., Ann. Rev. Biochem.
15 55:1091-1117 (1986)). It is also reasonable to predict that FKBP12A, FKBP12B and FKBP12C transcripts are regulated in a tissue-specific fashion, because the distribution of FKBP12B transcripts appears greater in heart, brain, placenta, and lung than in skeletal
20 muscle, kidney, liver, and pancreas (Figure 9). This variance could affect the immunophilin:calcineurin ratio postulated to be important for FK506- and CsA-specificity.

Similarly, from the elevated accumulation of
25 mature FKBP12A and FKBP12B transcripts in mitogenically stimulated T lymphocytes (Figure 8), it is reasonable to predict that one result of T cell activation could be increased demand for FKBP12 message, perhaps resulting in elevated FKBP12 levels. Of the three
30 FKBP12 transcripts, the FKBP12A message is the one most likely to be tightly regulated in a tissue-specific fashion, since it does not give rise to the FKBP12 processed pseudogenes (see DiLella, A.G. and Craig, R.J. 1991).

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The present invention also relates to isolated DNA, or RNA, having a nucleic acid sequence which is indicative of tissue-specificity of the mRNAs encoding FKBP12. At least three distinct messenger
5 RNAs in human cells can encode FKBP12. Three of these transcripts encoding FKBP12A, FKBP12B and FKBP12C, have been characterized as described above and have been shown to differ only in their 3'UTRs (Figure 3) and their predominant expression in specific mammalian
10 tissue. For example, FKBP12A is expressed in heart, placental and pancreatic tissues; FKBP12B, also found in these tissues, is additionally found in brain, lung, skeletal muscle, kidney and liver, in various quantities. Therefore, it is reasonable to predict
15 that the 3'UTR provides a useful marker sequence to determine tissue-specificity of FKBP12 mRNAs.

The present invention also relates to probes which hybridize to all or a portion of the 3'UTR of FKBP12s, and the use of these probes to detect the
20 presence of tissue-specific FKBP12 mRNAs. For example, one type of DNA probe is comprised of a DNA sequence complementary to the 3'UTR of FKBP12A mRNA. Thus, hybridization of the FKBP12A DNA probe to RNA present in a tissue sample would be useful to detect FKBP12A
25 mRNA present. Alternatively, an RNA probe could be used in a similar manner.

The present invention further relates to a method of monitoring rejection of transplanted tissue, or determining the effects of FK506 therapy to an
30 individual. Production of FKBP12 is essential to form the inhibitory complex of FK506/FKBP12. The FK506/FKBP12 complex acts as a potent immunosuppressant. If insufficient FKBP12 is produced in the target tissue to be effective in
35 immunosuppressive action, modifications of FK506

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therapy may be made or another line of therapy may be initiated, before tissue rejection or systemic toxicity occurs.

The present invention also relates to a
5 method of modifying production of FKBP12 in vivo. In the method, a nucleotide fragment, comprising either DNA or RNA complementary to DNA or RNA encoding FKBP12 is administered to an individual and hybridizes to all or a portion of the DNA or RNA encoding an FKBP. It is
10 reasonable to predict that hybridization could result in modified production of the FKBP. For example, the nucleotide sequence administered can be a DNA sequence complementary to the 3'UTR of FKBP12A mRNA. When the sequence hybridizes to the 3'UTR, translation of the
15 mRNA would be inhibited. Thus, production of FKBP12 in specific tissues such as heart, is decreased. A decreased production of heart FKBP12A could result in less competition for FK506 by heart tissue relative to, for example, kidney tissue. If a kidney had been the
20 transplanted organ, the immunosuppressant activity could be concomitantly increased in that specific tissue. Thus, in vivo modification of tissue-specific FKBP12 could increase effective immunosuppressant therapy for targeted tissues.

25 The present invention further relates to in vivo and in vitro methods of evaluating the effectiveness of an agent as an inhibitor of FKBP12 mRNA production and, thus, usefulness in immunosuppressive therapy. Measurement of an agent as
30 an inhibitor, may be accomplished by first, obtaining a baseline value of FKBP12 mRNA in cells a specific mammalian organ (i.e., the level of FKBP12 mRNA in the cells prior to or in the absence of treatment with an agent to be evaluated for its effectiveness as an
35 immunosuppressant) using nucleic acid probes which

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hybridize to all or a portion of the 3'UTR of FKBP12 mRNAs, as described above. The baseline value, is compared with the FKBP12 mRNA level in the same type of cells (e.g., a second sample or cells) after treatment with or in the presence of the agent being evaluated. Lower levels of FKBP12 mRNA after treatment with or in the presence of the agent (i.e., a post-treatment level of FKBP12 mRNA) is an indication that the agent inhibits FKBP12 mRNA production and, thus, can be used in immunosuppressive therapy. In an in vivo embodiment, the agent to be evaluated is administered to a mammal under conditions appropriate for the immunosuppressive agent to act upon production of FKBP12 mRNA. Subsequent to the administration of the agent, presence or absence or relative amounts of FKBP12 mRNA is determined using the nucleic acid probes of the method described. The difference between the baseline value and value obtained subsequent to administration of an agent shown to be an immunosuppressive agent, provides a reasonable basis of evaluating the effectiveness of the immunosuppressive agent in immunosuppressive therapy.

Alternately, cells may be isolated from mammalian tissue of interest, cultured in vitro, and a baseline value indicating the presence of FKBP12 mRNA in the cultured cells obtained as previously described. The cells are then treated with an agent to be evaluated as an immunosuppressant, under conditions appropriate for the agent to act upon production of FKBP12 mRNA in the cultured cells. As described, a lower FKBP12 mRNA level after treatment with or in the presence of an agent is an indication of its ability to inhibit FKBP12 mRNA production. Subsequent to the administration of the immunosuppressive agent, the presence or absence of FKBP12 mRNA is determined, with

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the difference between the two values providing a reasonable basis of evaluating the effectiveness of the agent in immunosuppressive therapy.

The present invention will now be illustrated
5 by the following examples, which are not to be seen as limiting in any way.

Example 1: Cloning and DNA Sequencing of FKBP-12A

Candidate FKBP12 cDNA clones were isolated from two human T lymphocyte cDNA libraries. A λ gt10
10 cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) of peripheral blood T cells stimulated with phytohaemagglutinin (PHA) for 48 hr was screened under nonstringent conditions with two synthetic oligomers derived from the open reading frame (ORF) sequence of
15 two FKBP12 cDNAs (Maki, N. et al., Proc.Natl. Acad. Sci. USA 87:5440-5443 (1990); Standaert, R.F. et al., Nature 346:671-674 (1990). The oligomers, (SEQ ID NOS: 6 and 7) were synthesized on an Applied Biosystems 380A DNA synthesizer and were radiolabeled with γ -³²P-
20 ATP. A full-length FKBP12 cDNA obtained from this screen (FKBP12A) was used to screen a second stimulated T lymphocyte cDNA library for additional FKBP12 cDNAs. The genomic clone λ VX18 was obtained by screening an EMBL-3 SP6/T7 library (Clontech) of human placental
25 genomic DNA with ³²P-labeled FKBP12A cDNA under stringent conditions. Genomic clones cVX5 and cVX8 were obtained from a pWE15 cosmid library (Stratagene Cloning Systems, La Jolla, CA) of human placental genomic DNA using hybridization probes under stringent
30 conditions. cVX5 was isolated with a 4.5 kb fragment of λ VX18 that extended from an Sfi I site just outside the SP6 promoter to the closest internal Kpn I site. cVX8 was isolated with a 764 bp fragment specific to the FKBP12B cDNA (generated by PCR using VX10230 and

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VX10232 as primers as shown in Figure 10) and was analyzed further after demonstrating that it also hybridized to a 319 bp Ban II/EcoR I fragment specific to the FKBP12 cDNA. The 738 bp cDNA insert of FKBP12A was excised by EcoR I (all restriction enzymes obtained from New England Biolabs, Beverly, MA) digestion and cloned into pUC19 for sequence analysis. Portions of the genomic clones containing sequences homologous to the FKBP12 cDNAs were identified by hybridization and cloned into pUC19 or pGEM®-3Z (Promega, Madison, WI) for sequence analysis. All DNA sequences were determined using α -³⁵S-dATP (Biggin, M.D. *et al.*, Proc. Natl. Acad. Sci. USA 80:3963-3965 (1983) and Sequenase® Version 2.0 DNA sequencing kits (USB, Cleveland, OH). Oligonucleotides were synthesized as required to serve as sequencing primers, and a commercial kit (USB) containing deoxy-7-deazaguanosine triphosphate was used to resolve compressions due to high G and C content (Mizusawa, S. *et al.*, Nucleic Acid Res. 14:1319-1324 (1986). Both DNA strands were sequenced completely a minimum of two times, and all sequences were analyzed with computer software from DNASTar, Inc. (Madison, WI). The FKBP12A cDNA sequence (SEQ ID NO: 1) has been deposited in GenBank with accession number X52220. The intron and exon sequences of the human FKBP12 gene have been deposited in GenBank with accession numbers M80706, M92422, M92423, and M93060.

Example 2: Southern Analysis of Human Genomic DNA

Human genomic DNA (Clontech Laboratories, Inc. Palo Alto, CA) was digested with the restriction endonucleases BamH I, BsaB I, EcoR I, Hind III, or Pst I and fractionated (10 µg per lane) in 1% agarose. The DNA was transferred to GeneScreen (New England Nuclear Research Products, Boston, MA) by capillary transfer

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using standard conditions and was crosslinked to the membrane with ultraviolet light (Stratalinker, Stratagene Cloning Systems, La Jolla, CA). The DNA probes were radiolabeled using α -³²P-dCTP and a commercial labeling kit (Pharmacia LKB Biotechnology, Piscataway, NJ) and were hybridized at 65°C under stringent conditions. The washed membranes were exposed at -70°C to X-Omat AR x-ray film (Eastman Kodak Company, Rochester, NY) using Cronex Lightning Plus (E.I. DuPont de Nemours and Company, Wilmington, DL) intensifying screens.

As shown in Figure 4, the hybridization probes were as follows: Lane 1 = 319 bp fragment specific to FKBP12A, generated by Ban II/EcoR I digestion of FKBP12A; Lane 2 = 524 bp fragment specific to FKBP12B and FKBP12C generated by PCR amplification of genomic DNA with primers VX10169 (SEQ ID NO:27) and VX10173 (SEQ ID NO:29); Lane 3 = 363 bp fragment, spanning the entire FKBP12 ORF, generated by Dde I digestion of FKBP12A DNA. DNA size markers are shown in kb and were determined by the migration profile of lambda DNA digested with EcoR I and Hind III.

Example 3: Characterization of the FKBP12 Gene and Chromosome Location

Using radiolabeled FKBP12A as a hybridization probe, a lambda library of human placental genomic/DNA was screened at high stringency. Four clones were selected and purified, and DNA from each clone was digested with restriction endonucleases and examined by Southern analysis. All clones hybridized to probes from the ORF, but they exhibited variable hybridization to probes from the 3' termini of the different FKBP12 cDNAs. One clone hybridized specifically to probes derived from the 3'UTRs of FKBP12B and FKBP12C, and sequence analysis indicated this clone was a processed

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pseud gene. The three remaining clones hybridized specifically to probes from the 3'UTR of FKBP12A and were extremely similar by restriction enzyme analysis. One of these clones, λ VX18, was analyzed further and
5 proved to be a large portion of the FKBP12 gene.

Mapping studies showed that the λ VX18 insert was approximately 15 kb in size and contained FKBP12A sequences localized at one end (Figure 5). Subcloning and sequence analysis revealed that the clone contained
10 two exons (Exons 3 and 4) separated by a 3.2 kb intron and corresponding to the ultimate 616 bp of the FKBP12A cDNA. Exon 3 contained 113 bp of ORF while Exon 4 contained 126 bp of ORF and the 377 bp FKBP12A 3'UTR (Figure 6). Exon 3 was also contained within the
15 FKBP12B and FKBP12C cDNA sequences, but only the first 165 bases of Exon 4 (the 126 bp of ORF, the TGA stop codon, and the next 36 bp) were found in these cDNA sequences (Figure 6). Additional library screens were performed to isolate genomic clones containing the 5'
20 exons of the FKBP12 mRNAs and the 3' exons of the 12B and 12C mRNAs.

Next, a cosmid library with inserts averaging 38 kb in size was screened. Two independent screening approaches were used: (1) cosmid selection with a
25 fragment from the 5' end of λ VX18 and (2) cosmid selection with a DNA fragment from the 3'UTR of FKBP12B followed by secondary selection with a DNA fragment from the 3'UTR of FKBP12B followed by secondary selection of a clone containing 5' end of the FKBP12
30 gene; the second approach assumed linkage of the genomic sequences encoding the different FKBP12 cDNAs.

Clone cVX5 was isolated by the first approach and contained the 5' end of the FKBP12 gene; clone cVX8 was isolated by the second approach and contained the
35 entire FKBP12 gene (Figure 5). Subcloning and

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sequencing part of cVX5 revealed two exons (Exons 1 and 2) separated by an 80 bp intron. These exons encoded the 5'UTR and first 85 bp of ORF in each FKBP12 cDNA. Specifically, Exon 1 contained the upstream sequence and first 37 bp of ORF while Exon 2 encoded the next 48 bp of ORF (Figure 6). Subcloning and sequencing part of cVX8 identified Exon 5 as the exon encoding the 3' ends of the FKBP12B and 12C mRNAs. DNA sequence from cVX8 also demonstrated directly that the 3'UTR of the FKBP12A transcript lies within the intron between Exons 4 and 5 and is generated by read through of the 3' splice junction of Exon 4 (see Figures 5 and 6). In conjunction with mapping studies of cVX5 and λ VX18, restriction and hybridization analysis of cVX8 indicated that the FKBP12 gene spans between 17-30 Kb.

Using PCR amplification to analyze DNA from human-hamster somatic cell lines representing the entire complement of human chromosomes, it was shown that amplification specific to the FKBP12 gene occurred only with DNA isolated from cell lines that contained human chromosome 20.

Human genomic DNA, genomic DNA from a Chinese hamster ovary cell line, and DNA from human-hamster hybrid somatic cell lines was obtained from Bios Corporation (New Haven, CT), and each was tested by PCR analysis for a sequence specific to the FKBP12 gene. The synthetic oligomers VX10027 and VX10247 (SEQ ID NOS: 8 and 9 respectively) served as the 5' and 3' primers, respectively. Each primer was used at a final concentration of 10 μ M in conjunction with 50 ng of each cell line DNA, and the total reaction volume was 50 μ l. Following 40 rounds of amplification (94°C, 1.5 min; 50°C, 1.5 min; 72°C, 3 min) on a MicroCycler E (Eppendorf, Fremont, CA), 10 μ l of each reaction was fractionated by horizontal gel electrophoresis in 3%

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NuSieve agarose (FMC BioProducts) and visualized with ethidium bromide. The amplified DNA fragments were transferred and crosslinked to nylon membrane, and the membrane was probed with two ^{32}P -radiolabeled oligomers
5 VX10171 and VX10174 (SEQ ID NOS: 10 and 11 respectively) internal to the amplified portion of the FKBP12 gene to confirm the identity of the amplified fragment. The amplified product was of the expected size (395 bp) and hybridized to synthetic DNA oligomers
10 derived from the gene sequence and internal to the PCR primers.

Example 4: RNA Preparation, Northern Analysis and PCR Amplification from RNA

Fresh human peripheral blood lymphocytes
15 (PBLs) were obtained from LeukoPaks (Children's Hospital Blood Bank, Boston, MA) derived from random, normal blood donors. The lymphocytes were isolated and separated by density centrifugation over Histopaque-1077 (Sigma Chemical Co., St. Louis, MO) according to
20 the manufacturer's instructions. The population was enriched for T lymphocytes by rosetting with sheep red blood cells for 1 hr at 4°C followed by another centrifugation through Histopaque-1077. Rosetted cells were recovered from the pellet after treatment with
25 0.16 M NH_4Cl , 0.01 M KHCO_3 , 0.1 mM EDTA, pH 7.4. Erythrocyte receptor positive PBLs (E^+ PBLs) were either harvested immediately or were cultured at 37°C at a concentration of 10^7 cells ml^{-1} in RPMI 1640 (Gibco BRL, Grand Island, NY) containing 50 units ml^{-1}
30 penicillin, 50 $\mu\text{g ml}^{-1}$ streptomycin, 2 mM L-glutamine, 50 μM β -mercaptoethanol, 10% heat-inactivated fetal calf serum, 10 mM HEPES pH 7.3 (Gibco BRL), and 10 $\mu\text{g ml}^{-1}$ PHA (Sigma) for 16 hr. All cells were then harvested at 250 x g for 10 min at room temperature and
35 washed twice with Hank's balanced salt solution (Gibco

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BRL). Total RNA was obtained by resuspending the washed cell pellet in 5-10 times its volume in 4.0 M guanidine thiocyanate, 0.1 M Tris-HCl pH 7.5, 1% β -mercaptoethanol and extracting as described by
5 Sambrook et al. (1989). Cytoplasmic RNA was purified as described by Gilman (1989). Poly(A)+RNA was selected with an mRNA spin column kit (5 Prime-3 Prime, Boulder, CO), resuspended in ribonuclease-free water, and stored at -70°C until use.

10 Poly(A)+RNA for Northern analysis was fractionated in 1% agarose containing formaldehyde before being transferred and crosslinked to nylon membrane as described above. The multiple tissue Northern blot was purchased from Clontech. DNA probes
15 were radiolabeled with α -³²P-dCTP as described above while RNA probes were transcribed from fragments cloned into pBluescript® II (Stratagene Cloning Systems La Jolla, CA). The membranes were hybridized, washed, and exposed to x-ray film as described from Southern
20 analysis. As shown in Figure 7A, Lane 1 = 20 hr exposure of the membrane; Lane 2 = 44 hr exposure. Marker sizes were determined by the migration profile of lambda DNA digested with EcoR I and Hind III and are indicated in kb.

25 RNA was prepared for polymerase chain reaction (PCR) analysis (Mullis, K.B. and Faloona, F.A. Methods Enzymol. 155:335-350 (1987); Saiki, R.K. et al., Science 239:487-491 (1988) using a modification of the procedure described by Brenner, C.A. et al.
30 Bio/Techniques 7:1096-1103 (1989). Briefly, cytoplasmic RNA was primed with oligo-dT₁₂₋₁₈ (Collaborative Research, Bedford, MA) and transcribed at 42°C for 1 hr with Moloney murine leukemia virus reverse transcriptase (BRL, Bethesda, MD) before being
35 used for PCR reactions. DNA products visible by

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ethidium bromide staining after thirty rounds of amplification were obtained using the first-strand cDNA produced from 0.17-1.3 μ g of total cytoplasmic RNA. DNA oligomers used to amplify specific portions of the different FKBP12 mRNAs were as follows: VX10172 (SEQ ID NO:28)-VX10247 (SEQ ID NO:9) for FKBP12A mRNA, VX10027 (SEQ ID NO:8)-VX10173 (SEQ ID NO:29) for FKBP12B mRNA, and VX10172 (SEQ ID NO:28)-VX10028 (SEQ ID NO:26) for the ORF common to all FKBP mRNAs (see Figure 10). A Perkin Elmer Cetus GenAmp 9600 was used for all PCR reactions that involved cytoplasmic RNA, and the DNA products were fractionated on 2% NuSieve 3:1 (FMC BioProducts, Rockland, ME) agarose gels. As shown in Figure 7B, Lane 1 = VX10172 (SEQ ID NO:28)-VX10028 (SEQ ID NO:26): specific to the ORF of all FKBP12 mRNAs, 299 bp fragment expected and obtained; Lane 2 = VX10172 (SEQ ID NO:28)-VX10247 (SEQ ID NO:9): specific to FKBP12A mRNA, 683 bp fragment expected and obtained; Lane 3 = VX10027 (SEQ ID NO:28)-VX10173 (SEQ ID NO:29): specific to FKBP12B mRNA, 607 bp fragment expected and obtained; Lane 4 = VX10201 (SEQ ID NO:30)-VX10801 (SEQ ID NO:33): specific to FKBP12C mRNA, 608 bp fragment expected but not obtained; Lane 5 = control primers SEQ ID NOS: 12 and 13, respectively to produce a 335 bp fragment specific for β 2-microglobulin.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Vertex Pharmaceuticals, Inc.
- (ii) TITLE OF INVENTION: METHOD OF DETECTING TISSUE-SPECIFIC
FK506 BINDING PROTEIN MESSENGER RNAs AND USES THEREOF
- (iii) NUMBER OF SEQUENCES: 33
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: c/o Fish & Neave
 - (B) STREET: 1251 Avenue of the Americas
 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 10020
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 07/886,611
 - (B) FILING DATE: 20-MAY-1992
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Haley Jr., James F.
 - (B) REGISTRATION NUMBER: 27,794
 - (C) REFERENCE/DOCKET NUMBER: VPI92-01 (PCT)
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (212) 596-9000
 - (B) TELEFAX: (212) 596-9090

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 738 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGGCGGCGGC GCCTCAGGTC CGGCGGCGGC ATGGGAGTGC AGGTTGAAAC CATCTGCGCA	60
GGAGAGGGGC GCACTTTCOC CAAGCGGGGC CAGACCTGGG TGGTGCCTA CACGGGGATG	120
CTTGAAGATG GAAAGAAATT TGATTCTCTC CGGACAGAA ACAAGGCTT TAAGTTTATG	180
CTAGGCAAGC AGGAGGTGAT CCGAGGCTGG GAAGAAGGGG TTGCGCAGAT GAGTGTGGGT	240
CAGAGAGCCA AACCTACTAT ATCTCCAGAT TATGCTATG GTGCGACTGG GCACTCAGGC	300
ATCATGCCAC CACATGCCAC TCTGCTCTC GATGTTGAGC TTCTAAACT GGAATGACAG	360
GAATGGCTC CTGCTTACG TCCCTGTTCT TGGGTAGGA AATGGAATAC TGAAGGGGCG	420
TTTACCTGCT TGTCTCTCTC CATGTTATGC CCAGGCTTGG ATGGGTAGCA GAGAGAACA	480
AAAACACCAC AAGGCTATTT TTGCGCTGC ATCTTTCTG TATGAGTAT CTTTTCAGTG	540
TTATTAGTGT ATGCTTTGAA TGTAAAAATT GGTACGCTA AGGAAAGCA TTGGCATGTG	600
TATGTTGCGA GTTCAACTCA TGGAGTGGC AGCTGTTTAA ATGTTTTCT ATGTAGTTTA	660
TAAATTAAAA CTAATTGAG GACTATGGAA ATGTAGGCGA ATTGTGTAGT GCGAATTTT	720
TATTTCTTGG GAAAAAAA	738

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 374 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAGGAATGGC CTCTCTCTT AGCTCTCTGT TCTTGGGTAA GGAATGGAA TACTGAGGG	60
CCCTTCACAG CCTTTGCTCT TCCATGTTA TTGCGAGCT TGTATGGTAA GCAGAGAGAA	120
CAAAAACAC CACAAGGCTA TTTTTCGCG TGCATCTTT CIGTATGAG TATCTTTCA	180
GIGTATTAG TGTATGCTTT GAATGTAAA ATTTGTCAC CTAAGGAAAG GAATTTGGAT	240
GIGTATGTC CCAGTTCAC TATGGAGAT GGCAGCTGT TAAATGTTT TCTATGTAGT	300
TTATAATTA AAATGAAAT GAGGACTATG GAAATGTAGG CCAATTTGT AGTCCACAA	360
TTTTAGTCT TTGG	374

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1532 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTGGGGC GGGGGGACAG TGGCTGTGTG TOCAGGCGGC GGGTGGGGGC GGGGGGGGGC	60
TCAGGGTGGC GGGGGGGCAT GGGAGTGCAG GGGGAAACA TCTGGGAGG AGAGGGGGGC	120
ACCTTGGGCA AGGGGGGACA GAGCTGGGTG GTGCACTACA CCGGATGCT TGAAGATGGA	180
AAGAAATTGT ATTCTGGGCG GGACAGAAAC AAGGCGTTTA AGTTTATGCT AGGCAAGCAG	240
GAGGTATGTC GAGGCTGGGA AGAGGGGGTT GGGCAGTGA GTGTGGGTCA GAGAGGAAA	300
CTGACTATAT CTGCAGTTA TGCTATGCT GGCATGGGC ACCAGGCAT CATGGGACA	360
CATGCGCTTC TGCTCTGGA TGTGGAGCTT CTAAACCTG AATGACAGGA ATGGGCTGCT	420
CGCTAGCTTC CGCTCTCTG GATCTGGCAT GGAGGGATCT GGCTGGTCCA GACATGTGCA	480
CATGAGTCCA TATGGAGCTT TTCTGATGT TOCCTGAC TTTGTATAGA CATCTGGCT	540
GACGATGTGT GTCTGTGAC TCAGCTTTC TTGGACAC TCCTGTTGCT CTGGGCTTT	600
CTCTGCTAT GTGTGTGAC CTAAACCTA TGCTATAAC CTCAAGTTAT TCATTTTATT	660
TTGTGTTTAT TTGGGGTGA AGATTCAGTT TCAGCTTTT GGATATAGGT TTCAATTAA	720
GTACATGGTC AAGTATTAC AGCACAAGTG GTAGGTAAAC ATTAGAATAG GAATGGGTGT	780
TGGGGGGGGG GTTGGCAAGA ATATTTTATT TTAATTTTTT GGATGAAAT TTTATCTATT	840
ATATATTAAA CATCTGTGCT GCTGGGCTGC AAAGCATAG CAGATTTCAG GGGCTGTGCA	900
GGACTGAATT ACTCTGCAAG TTGAGAGATG TCTTTGGGTT AAATTAAAG CCTACCTAA	960
AACAGAGGTG GGGATGGGGA GAGCTTTTC CTGACCATT CCGACGAC CTGGGCTTAA	1020
ACCTCTGGC TTGAAAGTA GATCATGTC ACTGCAATG TGGACACTAC AGGTATCTGT	1080
CGCTGGGACA GAGGGGACT CTAAGGCTT CTCTGGGCT TTTTTTTTTT TTCTATCTGT	1140
GGTTTCTTCA ATGGACTTC AGGAATTTG TAATCTATA ACTTTCAG CTGACCAT	1200
TCCTAAATCT TAAGACTTT AATGACAGT TTCAATTGAA GGCTCTGTT GTAGACTTAA	1260
CACGAGTGA AAGGCGAGC ATCATGACA ATCTGTGAT GTCTCTTAA GAAATGATG	1320
CTGTGATGTC GAGCTCAGC ATCTGCTGT TTTGATGCT TGGCTGGCT TGTGATCTC	1380

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AGTTTTCCTGG CTTTTCCTOC CTCAGGCGCT TCTACCGCT TTTCTGTCT GGTATGAT 1440
 TTGGTGAGAA ATGTTTCTG CAGGCTTTC CAGGCGCT TTTATGATCT CAGTTTAT 1500
 TATTGCAATA AAGTGCTTT ATGCGGAT TC 1532

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 583 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGCGCGAT GGGAGTGCAG GGGGAAACA TCTGCGAGG AGAGGGGCGC ACGTTCGCA 60
 AGGCGGCGCA GAGCTGGTG GGTGCTTACA CCGGATGCT TGAAGATGCA AAGAAATTG 120
 ATTCTTCGCG GACAGAAAC AAGGCTTTA AGTTTATGCT AGGCAAGCAG GAGGTATCC 180
 GAGGCTGGCA AGAGGGGTT GCGAGATGA GGTGGGCTA GAGAGCGAA CTGCTATAT 240
 CTCAGATTA TGCTATGCT GCGCTGGGC ACGGCGAT CATGCGCA CATGCGCTC 300
 TGTCTTGA TGTGGAGCT CTAAACTGG ATGACAGGA ATGCGCTCT CCGTATCTC 360
 CCGTCTCTG CATCTGCGAT GAGGCGCT GGTGCGCA GACATGCTA CATGCTGCA 420
 TATGAGCTT TCTGATGT TCGCTGAC TTGTATGA CATCTGCGT GATGATGT 480
 GTCTCTGAC TCGCTTGC TCGGACAC TCGTCTCT CTTGCGCTT CTCCTGAT 540
 GTGTCTTAC CTAACTATA TCGCTAAC CTCAGTAT TCA 583

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Gly Val Gln Val Glu Thr Ile Ser Pro Gly Asp Gly Arg Thr Phe
 1 5 10 15

Pro Lys Arg Gly Gln Thr Cys Val Val His Tyr Thr Gly Met Leu Glu
 20 25 30

- 29 -

Asp Gly Lys Lys Phe Asp Ser Ser Arg Asp Arg Asn Lys Pro Phe Lys
35 40 45

Phe Met Leu Gly Lys Gln Glu Val Ile Arg Gly Trp Glu Glu Gly Val
50 55 60

Ala Gln Met Ser Val Gly Gln Arg Ala Lys Leu Thr Ile Ser Pro Asp
65 70 75 80

Tyr Ala Tyr Gly Ala Thr Gly His Pro Gly Ile Ile Pro Pro His Ala
85 90 95

Thr Leu Val Phe Asp Val Glu Leu Leu Lys Leu Glu
100 105

(2) INFORMATION FOR SEQ ID NO:6:

(i) **SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AACAAGCOCT TTAAGTTTAT G

21

(2) INFORMATION FOR SEQ ID NO:7:

(i) **SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) **SEQUENCE DESCRIPTION: SEQ ID NO:7:**

CATTAACCTTA AAGGGCTTGT T

21

(2) INFORMATION FOR SEQ ID NO:8:

(i) **SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTGGCTCTTGG ATGTTGGAGC

19

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTITGGCACIA CAAATTGGC CTAC

24

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCAATTCTCT TTCTTTAGGG

20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCGCTTCACT GCGTTTGGTC

20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTGGGGCTAC TCTCTCTTTC TGG

23

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCCTACATGT CTGATCCAC TTAA

24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAGACGGUGA GUAG

14

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCGGUCUCU GUCUCCUCAG

20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

- 32 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGGGCA

6

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ACAACGGGCA GUUG

14

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

UACAGUGGUC UUUUUCACAG

20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGAUGC

6

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCCCAGGUAU GCUU

14

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CUUCCUUGUU CUUUUCACAG

20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AUGAGU

6

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

UUUUGGGUAA GGAA

14

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AGADUUUUCG UUUUUAACAG

20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ADUUGC

6

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CATCGAAGAC GAGAGTGGC

19

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GAGGGATCTG GTGCTTCAG

20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAGTGCAGGT GGAAACCATC

20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CATCCCCACC TCAGTTTAG

20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCTCAGGCTC CGGGGGGGGC

20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GGGGGAAGA TTCAGTTCA G

21

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CACTACACAG GACAGCAAAG G

21

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TTTTTTTTT TTTTGAATA ACTTGAGG

28

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CLAIMS

1. A cDNA clone of mammalian origin encoding an FK506 binding protein which is expressed predominantly in heart tissue, placental tissue and pancreatic tissue.
2. The cDNA clone of claim 1, said clone being of human origin.
3. The cDNA clone of claim 1, said clone comprising all or a portion of the nucleic acid sequence of SEQ ID NO: 1.
4. The cDNA clone of claim 3, said clone consisting essentially of the nucleic acid sequence of SEQ ID NO: 1.
5. An isolated DNA of mammalian origin, said DNA comprising all or a portion of the nucleic acid sequence of SEQ ID NO: 1.
6. The isolated DNA of claim 5, said DNA consisting essentially of the nucleic acid sequence of SEQ ID NO: 1.
7. An isolated DNA which encodes FKBP12 and comprises an untranslated 3'UTR nucleic acid sequence, said sequence being indicative of tissue distribution of FKBP12 mRNA.
8. The isolated DNA of claim 7, said untranslated 3' UTR nucleic acid sequence indicating expression of FDKP12 in RNA by a specific tissue.

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9. An isolated DNA, said DNA comprising all or a portion of the nucleotide sequence of SEQ ID NO: 2.

10. The isolated DNA of claim 9, said DNA consisting essentially of the nucleotide sequence of SEQ ID NO: 2.

11. An isolated DNA of mammalian origin which hybridizes to a DNA or RNA fragment having a nucleic acid sequence indicative of tissue specific production of FKBP12 mRNAs.

12. The isolated DNA of claim 11, wherein said DNA hybridizes to a DNA or RNA fragment and comprises an untranslated 3' UTR nucleic acid sequence indicating expression of FKBP12 mRNA by a specific tissue.

13. The isolated DNA of claim 11, said DNA being of human origin.

14. The isolated DNA of claim 11, wherein said DNA hybridizes to all or a portion of the nucleotide sequence of SEQ ID NO: 2.

15. A method of detecting FKBP12 mRNAs expressed in a mammalian tissue, comprising the steps of:

- a) obtaining a sample of mammalian tissue;
- b) preparing the sample of mammalian tissue so as to render RNA present therein available for hybridization with complementary nucleic acid, thereby producing a treated sample;

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c) contacting the treated sample with a nucleic acid probe which hybridizes to the 3'UTR of FKBP12 mRNAs; and

d) detecting hybridization of the nucleic acid probe with RNA from the treated sample, wherein hybridization is an indication of the presence of FKBP12 mRNA.

16. The method of claim 15, wherein the nucleic acid probe hybridizes to all or part of the nucleotide sequence of SEQ ID NO: 1.

17. The method of claim 15, wherein the nucleic acid probe hybridizes to all or a part of the nucleotide sequence of SEQ ID NO: 2.

18. A method of monitoring rejection of transplanted mammalian tissue, comprising the steps of:

a) obtaining a sample of transplanted tissue;

b) preparing the sample of transplanted tissue so as to render RNA present in the sample available for hybridization with complementary nucleic acid, thereby producing a treated sample;

c) contacting the treated sample with a nucleic acid probe which hybridizes to the 3'UTR of FKBP12 mRNA; and

d) detecting hybridization of the nucleic acid probe with RNA from the treated sample, wherein hybridization is an indication of the presence of FKBP12 mRNAs.

19. A method of monitoring the effects of FK506 therapy in a mammal comprising the steps of:

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- a) obtaining a sample of mammalian tissue;
- b) preparing the sample of mammalian tissue so as to render RNA present therein available for hybridization with complementary nucleic acid, thereby producing a treated sample;
- c) contacting the treated sample with a nucleic acid probe which hybridizes to the 3'UTR of FKBP12 mRNA; and
- d) detecting hybridization of the probe with RNA from the treated sample, wherein the extent of hybridization is indicative of the extent to which FKBP12 mRNA is present in the treated sample and hybridization below a threshold level is indicative of insufficient FKBP12 mRNA level in the tissue.

20. A method of modifying production of FKBP in an individual, comprising administering to the individual a nucleic acid sequence which hybridizes to all or a portion of the 3'UTR of FKBP12 mRNA such that hybridization results in the modified production of FKBP12.

21. The method of claim 15, wherein the nucleic acid sequence is a DNA or RNA sequence which hybridizes to all or a portion of the nucleotide sequence of SEQ ID NO: 1.

22. An in vivo method of evaluating the effectiveness of an agent as an inhibitor of FKBP12 mRNA production in mammalian cells, comprising the steps of:

- a) obtaining a first sample of cells from a mammalian tissue of interest;

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b) preparing the first sample of cells so as to render RNA present in the sample available for hybridization with complementary nucleic acid, thereby producing a treated sample;

c) contacting the first sample of treated cells with a nucleic acid probe which hybridizes to the 3'UTR of FKBP12 mRNAs;

d) detecting hybridization of the nucleic acid probe with FKBP12 mRNA from the first sample of treated cells, thereby obtaining a baseline value of FKBP12 mRNA;

e) administering to the mammal the agent to be evaluated, under conditions appropriate for said agent to act upon mRNA production;

f) obtaining a second sample of cells from the mammalian tissue sampled in a);

g) preparing the second sample of treated cells so as to render RNA present therein available for hybridization with complementary nucleic acid, thereby producing a second sample of treated cells;

h) contacting the second sample of treated cells with a nucleic acid probe which hybridizes to the 3' UTR of FKBP12 mRNAs; and

i) detecting hybridization of the nucleic acid probe with FKBP12 mRNA from the second sample of treated cells, thereby obtaining a post-treatment level of FKBP12 mRNA, wherein a lower post-treatment level of FKBP12 mRNA than baseline value is an indication that the agent inhibits FKBP12 mRNA thereby identifying an inhibitor of FKBP12 mRNA production.

23. An in vitro method of valuating the effectiveness of an agent as an inhibitor of FKBP12

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mRNA production in mammalian cells, comprising the steps of:

- a) obtaining cells from mammalian tissue of interest and culturing said mammalian cells under conditions appropriate for growth;
- b) obtaining a first sample of cells from the cultured cells of step a);
- c) preparing said first sample of cells so as to render RNA present in the cells available for hybridization with complementary nucleic acid, thereby producing a first sample of treated cells;
- d) contacting the first sample of treated cells with a nucleic acid probe which hybridizes to the 3'UTR of FKBP12 mRNAs;
- e) detecting hybridization of the nucleic acid probe with FKBP12 mRNA from the first sample of treated cells, thereby obtaining a baseline value of FKBP12 mRNA;
- f) treating said cultured mammalian cells of step a) with the agent to be evaluated, under conditions appropriate for said agent to act upon mRNA production;
- g) obtaining a second sample of cells from the cultured mammalian cells of step f);
- h) preparing the second sample of cells so as to render RNA present therein available for hybridization with complementary nucleic acid, thereby producing a second sample of treated cells;
- i) contacting the second sample of treated cells with a nucleic acid probe which hybridizes to the 3' UTR of FKBP12 mRNAs; and
- j) detecting hybridization of the nucleic acid probe with FKBP12 mRNA from the second sample of treated cells, thereby obtaining a post-

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treatment level of FKBP12 mRNA, wherein a lower post-treatment level of FKBP12 mRNA than baseline value is an indication that the agent FKBP12 inhibits mRNA production, thereby identifying an inhibitor of FKBP12 mRNA production.

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FIGURE 1

CCGCCCCCGCTCAGCGTCCGCCCCGCCATGGGAGTGCAGGTGGAAACCATCTCCCCAG
GAGACGGGCGCACCTTCCCCAAGCGCGGCCAGACCTGCGTGGTGCACTACACGGGGATGCT
TGAGGATGGAAAGAAATTTGATTCTCCCGGGACAGAAACAAGCCCTTTAAGTTTATGCTA
GGCAAGCAGGAGGTGATCCGAGGCTGGGAAGAAGGGGTTGCCCAGATGAGTGTGGGTCAGA
GAGCCAAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTGGGCACCCAGGCATCAT
CCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCTAAAACCTGGAATGACAGGAATGG
CCTCCTCCCTTAGCTCCCTGTTCTTGGGTAAGGAAATGGAATACTGAAGGGCCCTTCACTG
CCTTTGCTCCTCCCATGTTATGCCCAGCGTTTGATGGGTAGCAGAGAGAAACAAAAACACC
ACAAGGCTATTTTTCCCCCTGCATTCTTTCTGTATTGAGTATCCTTTCAGTGTTATTAGTG
TATGCTTTGAATGTAAAAATGGTCACCCTAAGGAAAGGAATTGGCATGTGTATGTTCCCA
GTTCAACTCATGGAGATGGCAGCTGTTTAAATGTTTTTCTATGTAGTTTATAAATTAAAC
TGAATTGAGGACTATGGAAATGTAGGCCAAATTTGTAGTGCCAACATTTTAGTTCTTTGGA
AAAAAA

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FIGURE 2

CAGGAATGGCCTCCTCCCTTAGCTCCCTGTTCTTGGGTAAGGAAATGGAATACTGAAGGGCCCTT
CACTGCCTTTGCTCCTCCCATGTTATGCCCAGCGTTTGATGGGTAGCAGAGAGAACAAAAACAC
CACAAGGCTATTTTTCCCCCTGCATTCTTTCTGTATTGAGTATCCTTTCAGTGTTATTAGTGTAT
GCTTTGAATGTAAAAATTGGTCACCCTAAGGAAAGGAATTGGCATGTGTATGTTCCCAGTTCAAC
TCATGGAGATGGCAGCTGTTTAAATGTTTTTCTATGTAGTTTATAAATTAAAACTGAATTGAGGA
CTATGGAAATGTAGGCCAAATTTGTAGTGCCAACATTTTAGTTCTTTGG

	Dde	
	↑	
-72	GGGCGCCGCCAGGTGCTGTTGGTCCACGCCGCCGTCGGC	CGCCCCCGCCGCTCAGGTC CGCCGCCGCGGC
	-30	-9
M G V Q V E T I S P G D G R T F P K R G Q T C V		
1 ATGGGAGTGCAAGTGAACCATCTCCCAGGACGCGGCGCACCTTCCCAAAGCGCGGCAGACCTGCGT		
1		
1		
V H Y T G M L E D G K K F D S S R D R N K P F		
72 GTGCACTACACGGGATGCTTGAAGATGAAAGAATTGATTCCTCCGGGACAGAAACAAGCCCTTTA		
72		
72		
K F M L G K Q E V I R G W E E G V A Q↓M S V G Q		
143 AGTTATGCTAGGCAAGCAGGAGGTGATCCGAGGCTGGGAAGAAGGGTTGCCAGATGAGTGGGTGAG		
143		
143		
R A K L T I S P D Y A Y G A T G H P G I I P P H		
214 AGAGCCAACACTGACTATATCTCCAGATTATGCCTATGGTGCCACTGGGCACCAGCATCATCCACCACA		
214		
214		
A T L V F D V E L L K L E *		
285 TGCCACTCTCGTCTTCGATGTGGAGCTTCTAAAACCTGGAATGACAGGAATGGCCTCCTCCCTTAGCTCCCT		
285		
285		
Ban II		
↑		
356 GTTCTTGGTAAGGAAATGGAATACTGAAGGGCCCTTCACTGCCTTTGCTCCTCCCATGTTATGCCACGGC		
356ATCTGCCATGGAGGGATCTGGTGCCCTCCAGACATGTGCACATGATGCCATATGGAGCTTTTCC		
356		

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FIGURE 3 (continued)

12A 427 TTGATGGGTAGCAGAGAACAAAAACACCAAGGCTATTTTCCCTGCTCTCTCTGTATTTGAG
 12B 427 TGATGTTCCACTCCACTTTGTATAGACATCTGCCCTGACTGAATGTGTTCTGTCACTCAGCTTTGCTTCCG
 12C 427
 12A 498 TATCCTTTCAGTGTATTAGTGTATGCTTTGAATGTAAATGGTCAACCTAAGGAAGGAATTGGCATG
 12B 498 ACACCTCTGTTTCCCTCTTCCCTTTCTCCCTGATGTGTGTACCTAACTATATGCCATAAACCTCAAG
 12C 498
 12A 569 TGTATGTTCCAGTTCAACTCATGGAGATGGCAGCTGTTAAATGTTTCTATGTAGTTTATAAATTA
 12B 569 TTATTCATTTTATTTTGTTCATTTTGGGTGAAGATTCAGTTTCAGTCTTTTGGATATAGGTTTCCAAT
 12C 569
 12A 640 ACTGAATTGAGGACTATGGAAATGTAGGCCAAATTTGTAGTGCCAAACATTTTAGTTCTTTTGGG
 12B 640 TAAGTACATGGTCAAGTATTAAACAGCACAAAGTGGTAGGTAAACATTAGAAATAGGAATTTGGTGT
 12B 711 GGGTTTGCAAGAATAATTTTATTTTAAATTTTGGATGAAATTTTATCTATTATATATATTAACAATTCTTGC
 12B 782 TGCTGGCTGCAAGCCATAGCAGATTTGAGGCGCTGTGAGGACTGAATTAATCTCCAAAGTTGAGAGATG
 12B 853 TCTTTGGTTAAATTAAAGCCCTACCTAAACTGAGGTGGGATGGGAGAGCCCTTTGCTCCACCATTC
 12B 924 CCACCCACCTCCCTTTAAACCCCTCTGCCCTTTGAAAGTAGATCATGTTCACATGCAATGCTGGACACTACAG
 12B 995 GTATCTGTCCCTGGCCAGCAGGACCCTCTGAAGCCCTTCTTTGTGGCCCTTTTCTTTTTCATCCTGTGGT
 12B 1066 TTTTCTAATGGACTTTCAGGAATTTTGTAAATCTCATAAATTTCCAAAGCTCCACCCACTTCTTAAATCTTAAAG
 12B 1137 AACTTTAATTGACAGTTTCAATTGAAGGTGCTGTTGTAGACTTAACACCCAGTGAAGCCAGCCCATCAT
 12B 1208 GACAAATCCTTTGAATGTTCTCTTAAGAAATGATGCTGGTCAATCGCAGCTTCAGCATCTCCTGTTTTGA
 12B 1279 TGCTTGGCTCCCTCTGCTGATCTCAGTTTCTCTGGCTTTTCTCCCTCAGCCCTTCTCAGCCCTTTGCTGT
 12B 1350 CCTGTGTAGTATTTGGTGAGAAATCGTTGCTGCACCCCTTCCCCCAGCACCAATTTATGAGTCTCAAGTTTT
 12B 1421 ATTATGCAATAAAAGTGCTTTTATGCCCGGCTTTTCTCA_n

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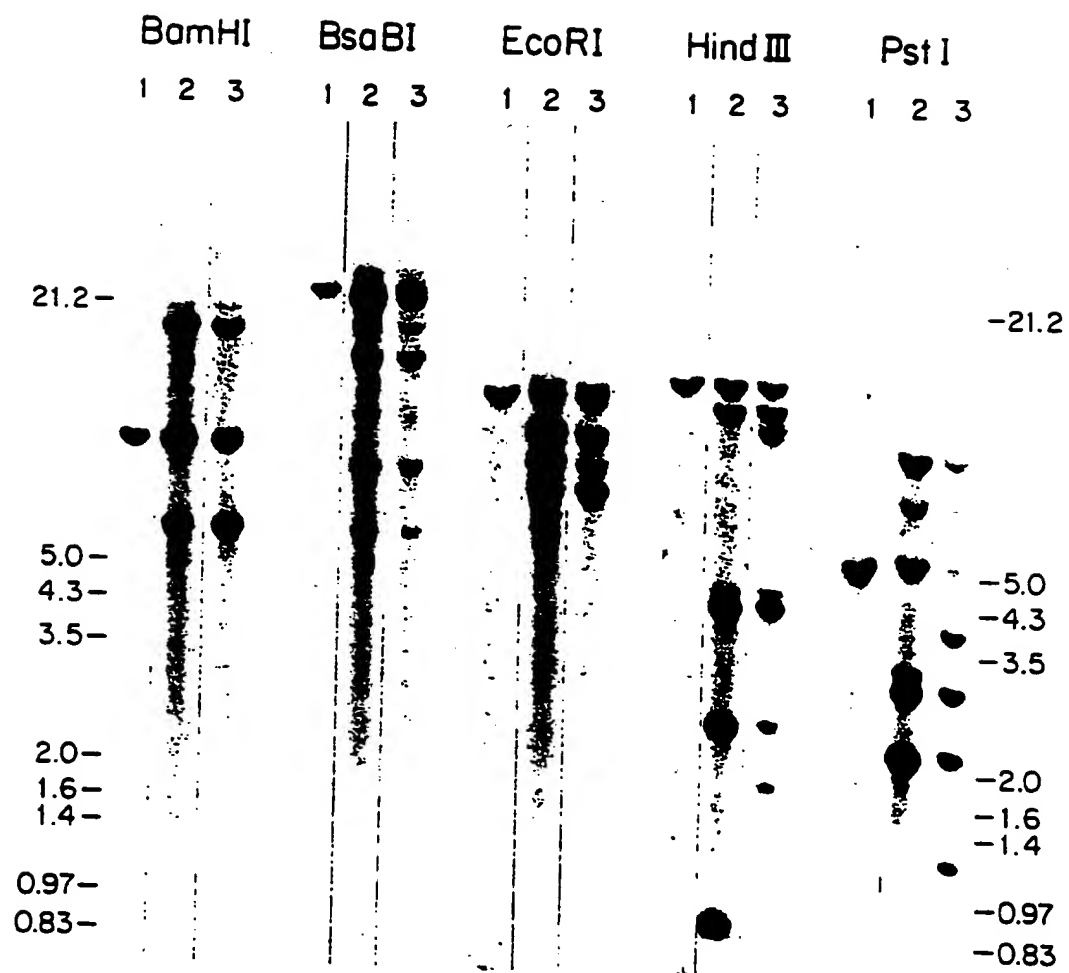
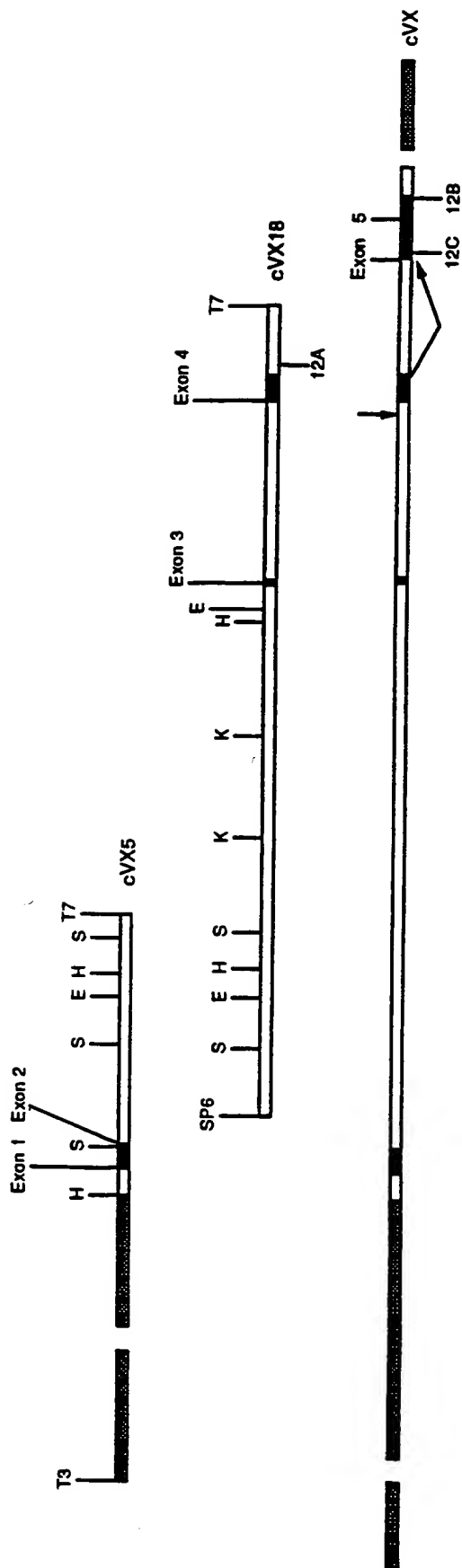


FIG. 4

SUBSTITUTE SHEET

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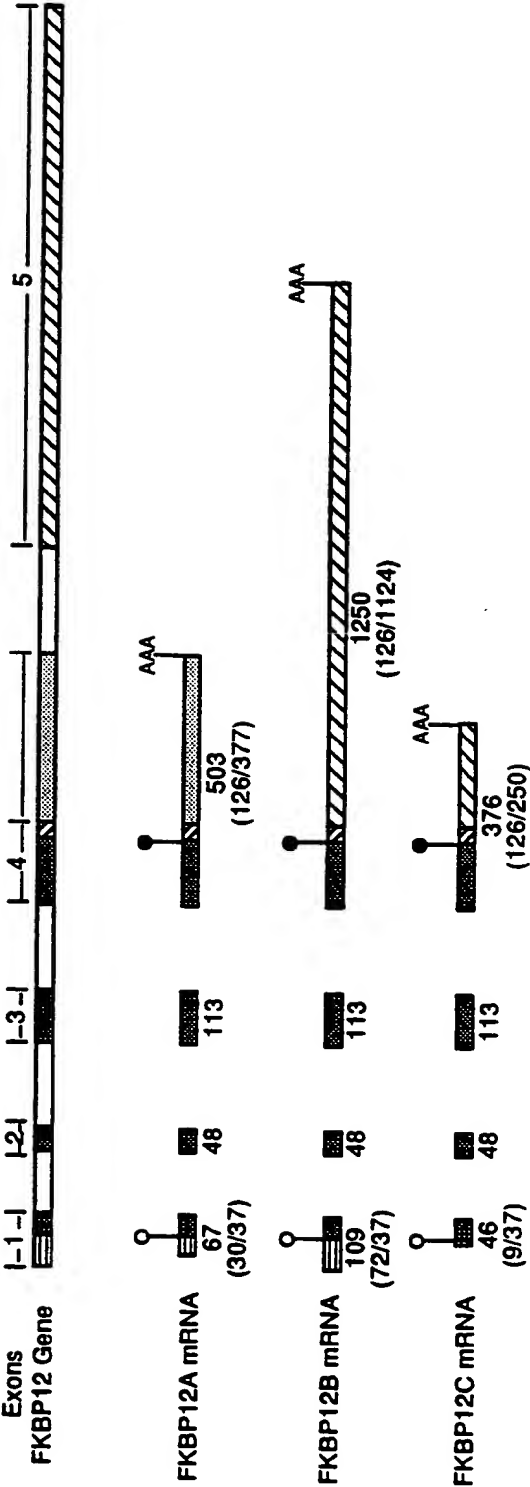


— = 1kb, except at indicated gaps

FIGURE 5

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FIGURE 6



	EXON	INTRON	EXON
Splice junction 1/2	GAGACG	GUGAGUAG...GCCCGUCUCUCUGUCUCCUCAG	(80 bp) GGCGCA
Splice junction 2/3	ACACCG	GUGAGUCG...UACAGUGGUCUUUUUACACAG	(~10 kb) GGAUGC
Splice junction 3/4	GCCCAG	GUAUGCUC...CUUCCUUGUUCUUUUUACACAG	(3.2 kb) AUGAGU
Splice junction 4/5	UCUUGG	GUAAGGAA...AGAUUUUCUGUCCCCAACACAG	(~2.2 kb) ATCTGC

Legend:
[] = 5' UTR of Exon 1
[] = ORF sequence
[] = Intron sequence
[] = 36 bp common element
[] = Intron sequence retained as exon sequence in FKBP12A mRNA
[] = Exon 5 used only in FKBP12B & FKBP12A mRNAs
O = ATG start
= TGA stop
AAA = poly(A) tail

FIG. 7A

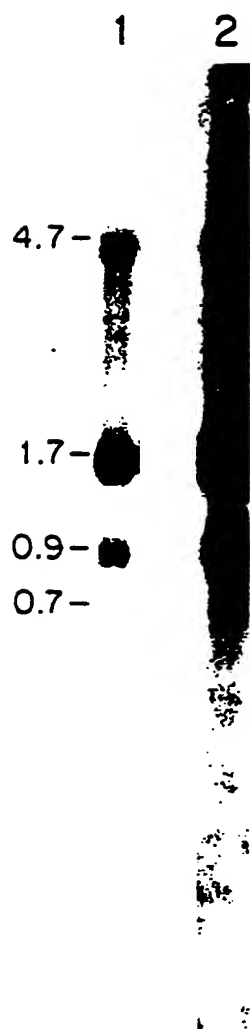
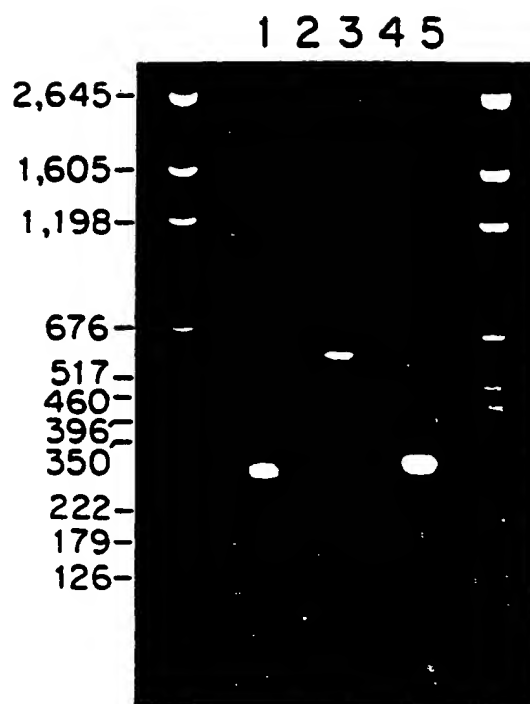


FIG. 7B



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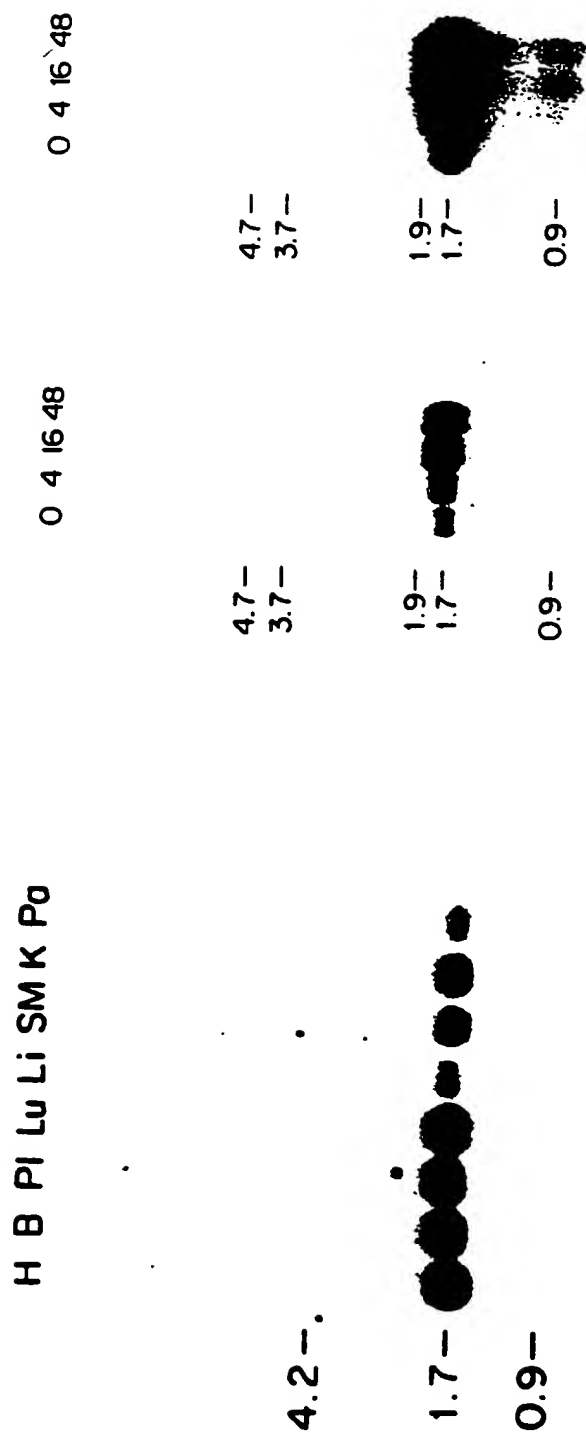
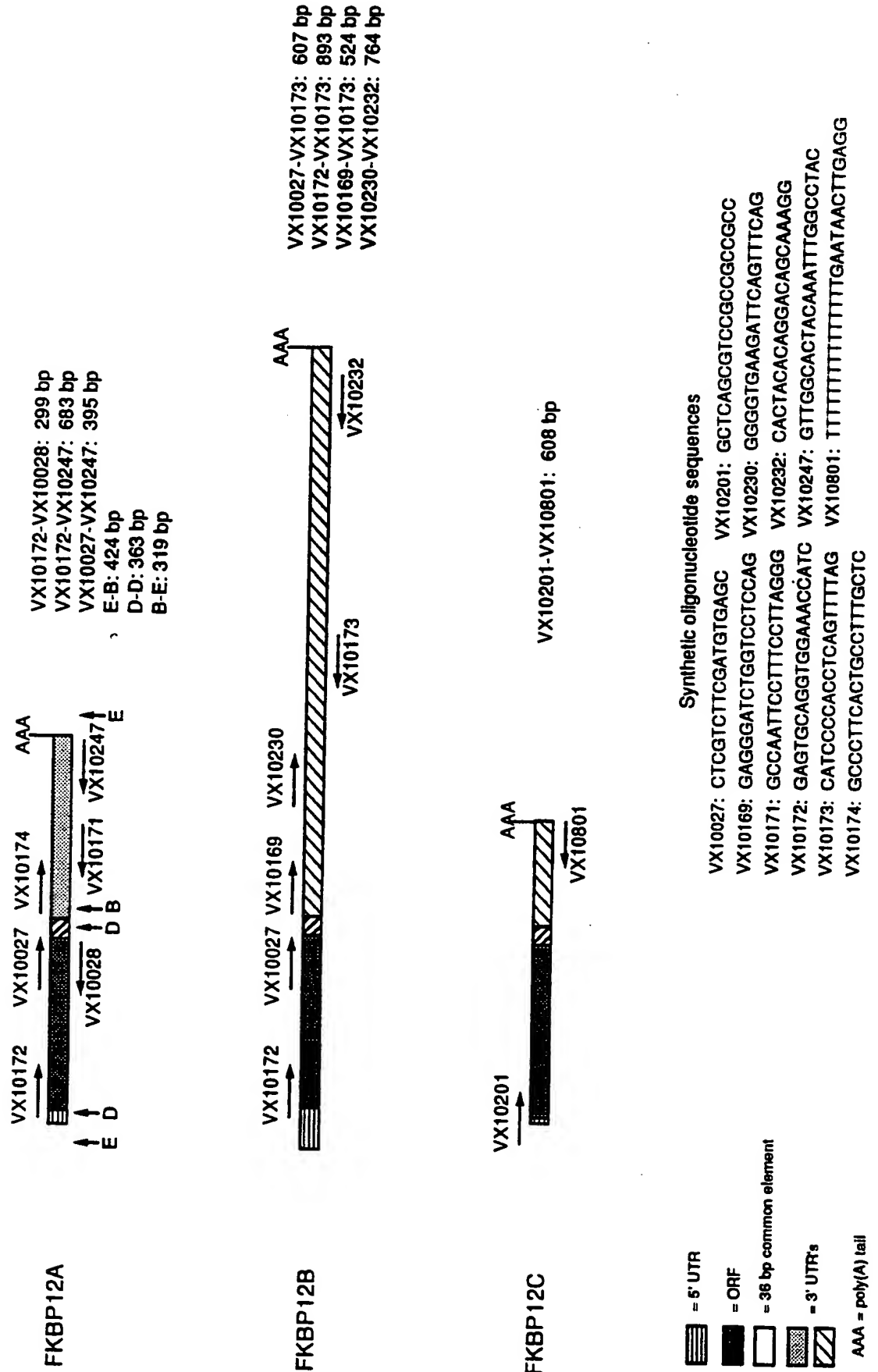


FIG. 8A FIG. 8B

FIG. 9

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FIGURE 10



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